

## <sup>1</sup> Abstract

- <sup>2</sup> 1. For many vertebrates, urban environments are characterised by frequent environmental stressors. Coping with such stressors can demand that urban individuals activate energetically costly physiological pathways more regularly than rural-living conspecifics. However, urban environments also commonly demand appreciable expenditure toward thermoregulation, owing to their often extreme climatic variation. To date, whether and how vertebrates can balance expenditure toward both the physiological stress response and thermoregulation, and thus persist in an urbanising world, remains an unanswered and urgent question among ecologists.
- <sup>10</sup> 2. We tested whether changes in body surface temperature ( $T_s$ ) and peripheral heat loss ( $q_{Tot}$ ) that accompany the stress response: 1) endow urban individuals with an enhanced capacity to conserve heat in the cold, and dissipate heat in the warmth relative to rural conspecifics, and 2) meet essential criteria for evolutionary responses to selection (here, variability among, and consistency within individuals).
- <sup>15</sup> 3. Using the black-capped chickadee ( $n = 19$ ) as a model species, we show that neither rapid nor chronic changes in  $T_s$  and  $q_{Tot}$  following stress exposure differed between urban- and rural-origin individuals ( $n_{urban} = 9$ ;  $n_{rural} = 10$ ). Nevertheless, we do find that stress-induced changes in  $T_s$  and  $q_{Tot}$  are highly repeatable across chronic time periods ( $R_{T_s} = 0.61$ ;  $R_{q_{Tot}} = 0.67$ ) and display signatures of stabilising or directional selection (i.e. reduced variability and increase repeatability relative to controls).
- <sup>21</sup> 4. Our findings suggest that, whilst urban individuals appear no more able to balance expenditure toward thermoregulation and the stress responses than rural conspecifics, the capacity to do so may still be subject to selection in chickadees.

<sup>24</sup> **Keywords;** Flexibility, Stress, Thermoregulation, Repeatability

<sup>25</sup> 1 | INTRODUCTION

<sup>26</sup> Over the past 70 years, the global human population has increased by approximately  
<sup>27</sup> 350% (or approximately 5.1 billion; United Nations, Department of Economic and  
<sup>28</sup> Social Affairs, Population Division 2019). Unlike in previous centuries, the major-  
<sup>29</sup> ity of individuals (nearly 54%) now reside in urban environments, and global trends  
<sup>30</sup> strongly suggest that urban living will increasingly become the norm (reviewed in  
<sup>31</sup> Lerch 2017). Consequently, land area designated for urban utility is expanding at  
<sup>32</sup> unprecedented rates and will probably continue to do so over the coming decades  
<sup>33</sup> (Angel *et al.*, 2011). Such expansion cannot, however, occur in a vacuum, and has  
<sup>34</sup> thus contributed to the widespread reduction in habitat availability and quality for  
<sup>35</sup> many species (Grimm *et al.* 2008; Seto *et al.* 2012; Freeman *et al.* 2019; lay literature:  
<sup>36</sup> Thomas 2017). For this reason, understanding whether these species can adapt and  
<sup>37</sup> persist within modern city-scapes has become a growing priority among modern  
<sup>38</sup> ecologists and conservationists (e.g. Birnie-Gauvin *et al.* 2016; Ouyang *et al.* 2018).

<sup>39</sup> Yet habitat loss or degradation are not the only challenges faced by species in ur-  
<sup>40</sup> ban environments. Indeed, urban environments regularly present acute challenges,  
<sup>41</sup> including noise, frequent human interaction, vehicle traffic, and in some cases, el-  
<sup>42</sup> evated depredation and inter- and intra-specific competition (Johnson *et al.* 2012;  
<sup>43</sup> Hernández-Brito *et al.* 2014; Newsome *et al.* 2015; Vincze *et al.* 2017; reviewed in  
<sup>44</sup> Lowry *et al.* 2013). Coping with these acute challenges can demand that individ-  
<sup>45</sup> uals within urban environments activate self-preserving physiological responses (i.e.  
<sup>46</sup> fight-or-flight responses) more regularly than rural-living conspecifics (Bonier 2012;  
<sup>47</sup> Watson *et al.* 2017; albeit, often with reduced intensity; Partecke *et al.* 2006; French  
<sup>48</sup> *et al.* 2008; but see Fokidis *et al.* 2009). Whilst such demands need not inherently trans-

<sup>49</sup> late to a loss of fitness among urban individuals, laboratory studies suggest that their  
<sup>50</sup> daily metabolic costs are probably raised owing to increased allostatic load (Depke  
<sup>51</sup> *et al.*, 2008; Jimeno *et al.*, 2017). In turn, these elevated metabolic demands may en-  
<sup>52</sup> hance susceptibility to wear and tear when resources are restricted or are required to  
<sup>53</sup> be allocated elsewhere (Romero *et al.*, 2009; Breuner & Berk, 2019).

<sup>54</sup> Beyond urban development, many of today's species face additional and indirect threats  
<sup>55</sup> associated with a growing human population. Effects of anthropogenic climate change  
<sup>56</sup> on species distribution and trait expression, for example, have now been argued for  
<sup>57</sup> nearly all taxa (e.g. Barton *et al.* 2016; Mainwaring *et al.* 2017; Pacifici *et al.* 2017;  
<sup>58</sup> Wan *et al.* 2018), and concerns over the ability of species to adjust to rising and in-  
<sup>59</sup> creasingly variable ambient temperatures (Vasseur *et al.*, 2014) have been well articu-  
<sup>60</sup> lated (e.g. Rutschmann *et al.* 2015; Radchuk *et al.* 2019). In endotherms, increases in  
<sup>61</sup> both maximal ambient temperature and variability of ambient temperatures can bear  
<sup>62</sup> notable thermoregulatory costs (Pendlebury *et al.*, 2004; du Plessis *et al.*, 2012; Smit  
<sup>63</sup> *et al.*, 2018), with those associated with the former being particularly severe in urban  
<sup>64</sup> environments (Arnfield, 2003). These costs, coupled with expected increases in sus-  
<sup>65</sup> ceptibility of wear and tear, beg important questions of whether and how endotherms  
<sup>66</sup> may cope with increasingly urbanised environments in the face of a rapidly changing  
<sup>67</sup> climate (discussed in Pautasso 2012; Argüeso *et al.* 2015; Brans *et al.* 2017).

<sup>68</sup> To date, several empirical studies have shown that endotherms may adjust their super-  
<sup>69</sup> ficial blood-flow, and thus, their body surface temperatures (henceforth, " $T_s$ ") when  
<sup>70</sup> exposed to stressors (e.g. Blair *et al.* 1959; Yokoi 1966; Nord & Folkow 2019; Winder  
<sup>71</sup> *et al.* 2020). In some species, these changes in  $T_s$  appear to endow individuals with  
<sup>72</sup> greater heat conservation in the cold, and greater heat dissipation in the warmth, thus

73 reducing their demands for costly thermogenesis or evaporative cooling respectively  
74 (Jerem *et al.* 2018; Robertson *et al.* 2020a; Winder *et al.* 2020). In this way, total en-  
75 ergetic expenditure may be balanced in challenging environments by allocating en-  
76 ergy toward more immediate and higher-cost threats (e.g. the perceived stressors)  
77 and away from less immediate and lower-cost threats (e.g. thermal challenges; Jerem  
78 *et al.* 2018; Robertson *et al.* 2020a). In urban environments, where individuals reg-  
79 ularly contend with both physical and thermal challenges, such flexibility of  $T_s$  and  
80 peripheral heat loss (here, non-evaporative heat-loss; henceforth, " $q_{Tot}$ ") could be par-  
81 ticularly advantageous, with those capable of enhanced flexibility (particularly during  
82 stress exposures) being better able to balance energy expenditure and, therefore, be-  
83 ing favoured by selection (Parsons, 2005). Nevertheless, the potential for selection to  
84 act on flexibility of  $T_s$  and  $q_{Tot}$  in response to stressors requires that these traits are  
85 both variable among individuals, and consistent within individuals (i.e. "repeatable";  
86 reviewed in Boake 1989; Wolak *et al.* 2012). Over the past two decades, numerous  
87 studies have reported moderate to high degrees of repeatability among traits associ-  
88 ated with the stress response and whole-animal metabolism (Nespolo & Franco 2007;  
89 Rensel & Schoech 2011; Müller *et al.* 2018; Boratyński *et al.* 2019; but see Ouyang  
90 *et al.* 2011). Whilst these finding strongly suggest that stress-induced changes in  $T_s$   
91 and  $q_{Tot}$  are also likely to be repeatable in endotherms, the degree of this repeatability  
92 remains largely unclear (but see Careau *et al.* 2012).

93 Using the black-capped chickadee (*Poecile atricapillus*; henceforth "chickadees") as a  
94 model species, we tested whether flexibility of both  $T_s$  and  $q_{Tot}$  during stress exposure:  
95 1) meet critical first criteria for responsiveness to selection, and 2) offer opportunities  
96 for endotherms to cope with the increased allostatic and thermoregulatory costs of  
97 an urbanising environment. More specifically, we hypothesised that stress-induced

98 changes in both  $T_s$  and  $q_{Tot}$ : 1) are variable among, and consistent within individu-  
99 als, 2) provide evidence of current or past selection, and 3) differ between individuals  
100 captured from urban and rural environments. Accordingly, we first predicted that  
101 stress-induced changes in both  $T_s$  and  $q_{Tot}$  would be repeatable among individuals.  
102 Because thermal responses to stress exposure can be acute (e.g. minutes to hours:  
103 Jerem *et al.* 2015; Andreasson *et al.* 2020; Winder *et al.* 2020) or chronic (e.g. days: de  
104 Aguiar Bittencourt *et al.* 2015; Herborn *et al.* 2018), and responses across each time-  
105 period may provide energetic benefits by enhancing heat dissipation or relaxing costs  
106 of thermogenesis (Jerem *et al.* 2018; Herborn *et al.* 2018; Winder *et al.* 2020; Robert-  
107 son *et al.* 2020a), we predicted that both acute and chronic changes in  $T_s$  and  $q_{Tot}$  ac-  
108 companying the stress response would be repeatable among individuals in our sample  
109 population. Next, because traits subject to previous or current selection (here, direc-  
110 tional or stabilising) are thought to display lower variability and higher repeatability  
111 than those that are selectively neutral (e.g. Gibson & Bradley 1974; Lande & Arnold  
112 1983; Boake 1989; Van Homrigh *et al.* 2007; but see Kotiaho *et al.* 2001), we predicted  
113 that both  $T_s$  and  $q_{Tot}$  of chickadees would be less variable and more repeatable dur-  
114 ing stress exposure treatments than during control treatments, after controlling for  
115 predictable environmental effects on heat loss (e.g. ambient temperature and relative  
116 solar radiation). Finally, because the combined energetic costs of stress exposure and  
117 thermoregulation are expected to be higher in urban environments when compared  
118 with rural environments (i.e. in the absence of phenotypic differences between urban  
119 and rural individuals; discussed above), we predicted that the magnitude of both acute  
120 and chronic changes in  $T_s$  and  $q_{Tot}$  that accompany stress exposures would be larger  
121 among urban-origin individuals than rural-origin individuals.

122 To test our predictions, we exposed chickadees captured from urban and rural envi-

123 environments to both repeated stressors and control conditions across an ambient temper-  
124 ature gradient whilst monitoring rapid and long-term changes in  $T_s$  and  $q_{Tot}$  by infra-  
125 red thermography. In small birds, surface tissues at the periorbital region (henceforth  
126 "eye region") are thought to play a critical role in environmental heat exchange (e.g.  
127 Hill *et al.* 1980; Powers *et al.* 2015) and both temperature of, and heat loss from this re-  
128 gion have previously been shown to respond to stress exposure (e.g. Jerem *et al.* 2015;  
129 Ikkatai & Watanabe 2015; Herborn *et al.* 2018; Robertson *et al.* 2020a). We, therefore,  
130 chose to use temperature of, and heat loss from, the eye region as our indicators of  $T_s$   
131 and  $q_{Tot}$  in this study.

132 The capacity of vertebrates to cope with the combined pressures of urbanisation and  
133 anthropogenic climate change has been questioned many times (Pautasso, 2012; Argüeso  
134 *et al.*, 2015; Brans *et al.*, 2017). The proximate physiological mechanisms by which  
135 vertebrates (here, endotherms) may do so, however, are seldom explored. Ours study,  
136 therefore, represents a critical step forward in how ecologists might test the capacity  
137 of vertebrates to adapt an increasingly human-modified world.

## 138 2 | MATERIALS AND METHODS

139 All methods used for animal capture, sampling, and experimental treatment were ap-  
140 proved by the Trent University Animal Care Committee (AUP # 24614) and Envi-  
141 ronment and Climate Change Canada (permit # 10756E).

## <sup>142</sup> 2.1 | Capture, transport, and housing of experimental animals

<sup>143</sup> Chickadees ( $n = 20$ ;  $n = 10$  females,  $n = 10$  males) used for this experiment were  
<sup>144</sup> captured within a  $100 \text{ km}^2$  region of south-western Ontario, Canada, between the  
<sup>145</sup> months of March and April in 2018. To minimise the possibility of kinship be-  
<sup>146</sup> tween individuals within our sample population, capture efforts were divided across  
<sup>147</sup> six distinct locations (three urban and three rural), each separated by a minimum  
<sup>148</sup> distance of 15 km. Urban capture locations included the downtown regions of the  
<sup>149</sup> cities of Brantford ( $43.1345^\circ\text{N}$ ,  $80.3439^\circ\text{W}$ ), Cambridge ( $43.3789^\circ\text{N}$ ,  $80.3525^\circ\text{W}$ ),  
<sup>150</sup> and Guelph ( $43.3300^\circ\text{N}$ ,  $80.1500^\circ\text{W}$ ), whilst rural capture locations included the town-  
<sup>151</sup> ships of Corwhin ( $43.5090^\circ\text{N}$ ,  $80.0899^\circ\text{W}$ ), Erin ( $43.7617^\circ\text{N}$ ,  $80.1529^\circ\text{W}$ ), and Cayuga  
<sup>152</sup> ( $42.9797^\circ\text{N}$ ,  $79.8745^\circ\text{W}$ ) (SFigure 1). A difference in the mean degree of urbanisation  
<sup>153</sup> between urban and rural capture locations was validated using methods similar to  
<sup>154</sup> Thompson et al (2018; see Appendix; SFigures 2–4).

<sup>155</sup> All individuals were captured using modified potter traps (dimensions [ $\text{L} \times \text{W} \times \text{H}$ ]  
<sup>156</sup> =  $90 \times 70 \times 70 \text{ cm}$ ), baited with sunflower seeds and suet on the day of capture.  
<sup>157</sup> To further draw individuals to trap locations, we alternately broad-casted chickadee  
<sup>158</sup> breeding songs and alarm calls from a remote call-box (FoxPro™ Patriot; Lewisville,  
<sup>159</sup> PA, USA) until at least one individual approached a potter trap by  $\leq 4$  meters. Upon  
<sup>160</sup> capture, chickadees were blood sampled (approximately  $50 \mu\text{L}$ ) by brachial venipunc-  
<sup>161</sup> ture and capillary tube collection, then fitted with one stainless steel, numbered leg  
<sup>162</sup> ring (size 0) and a unique combination of two, coloured, Darvic leg rings (size 0) for  
<sup>163</sup> future identification. Each individual was then measured (mass to the nearest 0.1 g  
<sup>164</sup> using an electronic scale, and, wing cord to the nearest 0.1 mm, left outer tarsus to  
<sup>165</sup> the nearest 0.1 mm, and head-to-bill to the nearest 0.1 mm using analogue calipers)

<sup>166</sup> and secured in a covered flight enclosure (dimensions  $[L \times W \times H] = 30 \times 30 \times$   
<sup>167</sup> 15 cm) for transportation to our long-term housing facility (Ruthven Park National  
<sup>168</sup> Historic Site, Cayuga, Ontario;  $\leq 90$  km drive). Blood samples were preserved in a  
<sup>169</sup> small volume of Queen's Lysis buffer ( $500 \mu\text{L}$ ; Seutin *et al.* 1991) for use in genetic  
<sup>170</sup> sexing (described in Robertson *et al.* 2020a) and were held on ice until storage at  $4^\circ\text{C}$   
<sup>171</sup> was possible ( $\leq 2$  hours).

<sup>172</sup> Upon arrival to our long-term housing facility, chickadees were haphazardly dis-  
<sup>173</sup> tributed among four, visually isolated flight enclosures ( $n = 5$  per enclosure; dimen-  
<sup>174</sup> sions  $[L \times W \times H] = 1.83 \times 1.22 \times 2.44$  m), each equipped with one white cedar tree  
<sup>175</sup> (*Thuja occidentalis*), two perching branches (raised to approximately 1.50 and 1.80 m  
<sup>176</sup> above ground) and a raised feeding platform ( $400 \text{ cm}^2$ ) at which food was provided  
<sup>177</sup> *ad libitum* through an opaque hinged door for the duration of the experiment (Fig-  
<sup>178</sup> ure 1). Food provided included sunflower seed, safflower seed, shelled peanuts, boiled  
<sup>179</sup> egg, apple pieces, house crickets (*Acheta domesticus*), meal worms (*Tenebrio molitor*)  
<sup>180</sup> and Mazuri (St Louis, MO, USA) Small Bird Maintenance diet. Water was also pro-  
<sup>181</sup> vided *ad libitum* across our experiment through opaque hinged doors. All individuals  
<sup>182</sup> were given a minimum of 2 weeks to acclimate to enclosures and social groups prior  
<sup>183</sup> to onset of experimentation.

## <sup>184</sup> 2.2 | Experimental stress exposure

<sup>185</sup> To test repeatability of stress-induced thermal responses within and among individuals,  
<sup>186</sup> we used a paired experimental design wherein each individual was exposed to both a  
<sup>187</sup> thirty-day control treatment and a thirty-day stress exposure treatment, with treat-  
<sup>188</sup> ments separated by an additional two-day control period (total experimental duration

<sup>189</sup> = 62 days). To control for possible effects of treatment order on stress-induced thermal  
<sup>190</sup> responses, half of our sample population ( $n = 10$  across two flight enclosures) was ex-  
<sup>191</sup> posed to control treatments followed by stress-exposure treatments, whilst the second  
<sup>192</sup> half of our sample population ( $n = 10$  across two flight enclosures) was concurrently  
<sup>193</sup> exposed to a reversed treatment order (i.e. stress-exposure treatments followed by  
<sup>194</sup> control treatments).

<sup>195</sup> Each day, individuals within stress exposure treatments were exposed to 5 or 6 exper-  
<sup>196</sup> imental stressors, with each being applied for 20 minutes and being separated from  
<sup>197</sup> previous and subsequent stress exposures by  $\geq 1$  hour (similar to Rich & Romero  
<sup>198</sup> 2005). Timing and type of experimental stressors were randomly selected each day  
<sup>199</sup> to minimise the potential for habituation to each given stressor type. Experimen-  
<sup>200</sup> tal stressors included the presence of a novel object (a garden gnome), presence of  
<sup>201</sup> a mock predator (a taxidermically mounted Cooper's hawk; *Accipiter cooperii*), cap-  
<sup>202</sup> ture and restraint in an opaque fabric bag, presence of a human, covering of a given  
<sup>203</sup> flight enclosure with an opaque tarp (simulating extreme, inclement weather), and  
<sup>204</sup> presence of a taxidermically mounted conspecific fixed to the feeding platform of a  
<sup>205</sup> given flight enclosure (simulating a novel, dominant individual). In a previous study,  
<sup>206</sup> chickadees exposed to our randomised stressor protocol displayed a significant reduc-  
<sup>207</sup> tion in feeding rate and mass, and regular evoked alarm calls (Robertson *et al.* 2020b),  
<sup>208</sup> providing strong support for protocol efficacy. Endocrine responses to stressor types  
<sup>209</sup> were not measured to circumvent effects of blood sampling on surface temperature  
<sup>210</sup> measurements and stress perception among sampled individuals. Individuals exposed  
<sup>211</sup> to control treatments were left undisturbed in an adjacent flight enclosure and blind  
<sup>212</sup> to experimenter presence.

213 Because flight enclosure were not auditorily segregated, estimated thermal responses  
214 to stress exposure in this study (i.e., the interaction between time or ambient temper-  
215 ature and treatment type) are expected to be conservative.

216 **2.3 | Infrared thermography, body surface temperature estima-**  
217 **tion, and heat transfer estimation**

218 We monitored  $T_s$  and  $q_{Tot}$  of chickadees indirectly using remote infra-red thermogra-  
219 phy (thermographic camera: FLIR VueProR<sup>TM</sup>, 13 mm, 226 × 356 resolution: accu-  
220 racy = ± 5%; image frequency = 1 Hz), as per Robertson et al (2020a). Specifically, we  
221 captured infrared thermographic images (radiometric JPEGs) of individuals at feeding  
222 platforms across the duration of our experiment from weather-proofed camera boxes  
223 mounted to the exterior of enclosure walls (0.5 m distance). To minimise temporal  
224 bias of thermographic imaging among social groups, we rotated our thermographic  
225 camera cardinally clock-wise among flight enclosures each day, with filming dura-  
226 tions persisting for approximately one hour per enclosure, and the first flight enclosure  
227 to receive thermographic filming being randomly selected on the morning of each  
228 day. Because leg-ring combinations could not be readily distinguished from thermo-  
229 graphic images, we also captured digital video (camera: Ion Air Pro<sup>TM</sup> 1014W, 1280  
230 × 720 resolution) of feeding individuals in parallel to themographic images to permit  
231 individual identification. All thermographic imaging and digital video used in this  
232 study were captured between 08:00h and 16:00h of each day.

233 Estimation of an object's  $T_s$ , and consequently rate of heat transfer by infrared ther-  
234 mography requires that local ambient temperature and relative humidity are known  
235 (Minkina & Dudzik, 2009; Tattersall, 2016). We therefore monitored ambient tem-

perature at enclosures subjected to thermographic filming using a ThermoChron iButton™ (Maxim Integrated, DS1922L-F5, San Jose, CA, USA) placed in the shade, at a frequency of 1 reading/5 minutes. Relative humidity readings were collected from a nearby weather station operated by Environment and Climate Change Canada (station identity = Hamilton A, 22 km from the experimental holding location) at the maximum available frequency of 1 reading/hour.

To estimate  $T_s$  from infrared thermographic images, we followed methods described by Robertson et al (2020a). Specifically, raw infra-red radiance ( $\text{kW/m}^2$ ) values per pixel were manually extracted in R statistical software (version 3.6.1; R Core Team 2019) then first converted to temperature ( $^\circ\text{C}$ ) per pixel according to Planck's law, ambient temperature and humidity estimates at the time of image capture, and equations outlined elsewhere (Minkina & Dudzik 2009; Tattersall 2016). Emissivity of the eye region of chickadees was assumed to be fixed at 0.95 according to estimates made for integument of Canadian and snow geese (*Branta canadensis* and *Chen caerulescens* respectively; Best & Fowler 1981). Following their estimation, temperature values per pixel were then integrated into FITS matrices using the R package FITSio (version 2.1.0; Harris 2016; one matrix per thermographic image), and eye region  $T_s$  values (here, maximum temperature values, as per Jerem *et al.* 2015) were manually extracted from within matrices using the open-sourced software FIJI (Schindelin *et al.* 2012; average size of eye region  $\approx 230$  pixels). To minimise underestimation of  $T_s$  as a consequence of image blurring, only values extracted from individuals that were stationary during image capture were included in our final data (Tattersall, 2016).

To estimate  $q_{\text{Tot}}$  ( $\text{mW}$ ) from  $T_s$  measurements, we followed equations described by McCafferty *et al.* (2011) and Nord & Nilsson (2019). Here, however, values for the

kinematic viscosity of air ( $\text{m}^2/\text{S}$ ; at an assumed atmospheric pressure of 101.325 kPa) and the thermal expansion coefficient of air ( $1/\text{K}$ ) were estimated for each given ambient temperature using the R packages "bigleaf" and "Thermimage" respectively (Knauer *et al.*, 2018; Tattersall, 2019). For this study,  $q_{\text{Tot}}$  was assumed to equal the sum of convective and radiative heat transfer, owing to both the minimal effects of wind-speed in our flight enclosures, and low likelihood of heat transfer between the eye region and any medium other than air during our experiment. Surface area of the eye region was estimated as  $0.864 \text{ cm}^2$  according to Robertson et al (2020a), and contours within the eye region were considered negligible. Final  $q_{\text{Tot}}$  estimates were multiplied by two to represent total rates of heat transfer across both eye regions.

## 2.4 | Statistical analyses

All statistical analyses were conducted in R software (version 3.6.1; R Core Team 2019 with each generalised additive mixed-effects model ("GAMM") constructed in the package "brms" (version 2.13.3; Bürkner 2017). Additionally, all models were run using Markov Chain Monte Carlo (MCMC) sampling, with 4 Markov chains, 10000 chain iterations, and 1000 warm-up iterations to maximise mixing and convergence of Markov chains. Final iterations were thinned by 10 to account for possible autocorrelation between MCMC draws, and models were validated by visually diagnosing residual distributions and trace plots.  $\hat{R}$  values for all model parameters fell between 0.99 and 1.01, and the ratio of effective sample sizes to our total sample size were greater than 0.65 for each parameter. Lastly, all figures were produced in R using the package "ggplot2" (version 3.3.2; Wickham 2016), and one individual (a female captured in an urban environment) was removed due to an unusually small sample size ( $n = 19$  thermographic images).

284 2.4.1 | Thermal responses to stress exposure among individuals

285 To first test whether acute and chronic changes in  $T_s$  accompanying the stress re-  
286 sponses were repeatable among individuals, we constructed two Bayesian hierarchical  
287 GAMMs wherein we estimated both global responses and individual-level responses  
288 to stress exposure across acute and chronic time-scales. In both models, tempera-  
289 ture of the eye region of individuals ( $^{\circ}\text{C}$ ; Gaussian distributed) was included as the  
290 response variable, and treatment type (i.e. stress exposure or control) and sex were  
291 included as linear, population-level predictors to account for the influence of each on  
292 eye region temperature measurements. Additionally, flight enclosure identity, date of  
293 thermographic image capture, and individual identity were included in each model as  
294 group-level intercepts to account for statistical non-independence between measure-  
295 ments collected from the same flight enclosure, day, and individual, and a group-level  
296 slope for time of day per flight enclosure orientation (i.e. east facing or west facing)  
297 was included to account for differential exposure to solar radiation within east- and  
298 west-facing enclosures across time.

299 In our model predicting acute thermal responses to stress-exposure, time post stress  
300 exposure (seconds), ambient temperature, and time of day (hour) were each included  
301 as population level predictors. Because acute, stress-induced changes in  $T_s$  at the eye  
302 region are thought to be non-linear (Jerem *et al.*, 2015, 2019), time post-stress ex-  
303 posure was included as a cyclic cubic regression spline with 5 knots fixed at -1200,  
304 0, 1200, 2400, and 3600 seconds to evenly distribute model fitting across each phase  
305 of stress exposure (i.e. before, during, and after exposure). Here, a cyclic regression  
306 spline was chosen to capture expected returns to baseline  $T_s$  (as reported for blue tits,  
307 *Cyanistes caeruleus*; Jerem *et al.* 2019) following 40 minute recovery periods. To per-

308 mit comparisons between stress exposed and control treatments, we paired enclosures  
309 such that time post stress exposure for an enclosure experiencing a control treatment  
310 was considered to be equivalent to that of the nearest enclosure experiencing a stress  
311 exposure treatment and equivalent cardinal orientation (i.e. west- or east- facing). As  
312 such, our comparisons between treatments account for indirect effects of experimental  
313 stress exposures on nearby control individuals.

314 In endotherms,  $T_s$  is expected to display non-linear relationships with both ambi-  
315 ent temperature and time of day owing to peripheral thermoregulatory processes  
316 (i.e. cold-induced vasoconstriction and warm-induced vasodilation) and circadian  
317 rhythms (Richards, 1971; Cooper & Gessaman, 2005) respectively. Ambient tempera-  
318 ture and time of day were therefore included as natural cubic and thinplate regression  
319 splines respectively, each with 4 knots to minimise risk of model over-fitting. Knots  
320 for our ambient temperature spline were evenly spaced by quantiles to uniformly cap-  
321 ture trends in eye region temperature at ambient temperatures below, within, and  
322 above thermoneutrality for our study species (Grossman & West, 1977). Because we  
323 did not have *a priori* assumptions for knot positions for our time of day spline, knot  
324 positions were chosen by truncated eigen decomposition (Wood, 2003). To control  
325 for differential effects of treatment type on  $T_s$  across time (Jerem *et al.*, 2015, 2019) and  
326 ambient temperature (Robertson *et al.* 2020a), population-level interactions between  
327 treatment type and ambient temperature, and treatment type and post stress exposure  
328 were also included as model predictors, along with an interaction between treatment  
329 type and the tensor product ( $\otimes$ ) between ambient temperature and time post stress ex-  
330 posure to account for the influence of ambient temperature on acute thermal responses  
331 to stress exposure at the skin (Nord & Folkow, 2019). All interaction terms were pe-  
332 nalised on the first derivative to minimise the potential for concurvity between inter-

333 action terms and main effects. Finally, to estimate differences in acute, stress-induced  
334 changes in  $T_s$  among individuals, group-level slopes for time post stress exposure and  
335 the interaction between time post-stress exposure and treatment type were included  
336 for each individual. Correlation between adjacent  $T_s$  measurements was corrected  
337 using a type-I auto-regressive (AR1) correlation structure with an estimated rho ( $\rho$ )  
338 of 0.69, and residual error was estimated independently for each treatment type.

339 In our model predicting chronic stress-induced changes in  $T_s$ , group-level predictors  
340 remained as described above but with minor adjustments. Specifically, all predictors  
341 including time post stress exposure (i.e. as a main effect or interactive effective) were  
342 excluded from our model to permit assessment of long-term, but not short-term trends  
343 in  $T_s$  according to treatment type. Furthermore, to estimate differences in chronic  
344 stress-induced changes in  $T_s$  among individuals, group-level slopes for ambient tem-  
345 perature and the interaction between ambient temperature and treatment type was  
346 included per individual. Here, ambient temperature was mean-centered and scaled to  
347 2 times the standard deviation (as per Araya-Ajoy *et al.* 2015) to allow for individual  
348 slopes to be estimated with respect to our average environmental conditions. Again,  
349 correlations between adjacent  $T_s$  measurements was corrected using an AR1 corre-  
350 lation structure ( $\rho = 0.69$ ), and residual error was estimated separately per treatment.

351

352 Because rates of peripheral heat transfer ( $q_{Tot}$ ) are proportional to  $T_s$  at given ambient  
353 temperatures, both acute and chronic changes in  $q_{Tot}$  accompanying stress exposure  
354 treatments were modeled as described above. In these models, however,  $q_{Tot}$  was used  
355 as the response variable (mW; Gaussian distributed) in place of  $T_s$ .

356 In all hierarchical models, we used informed priors for our population intercept, our  
357 coefficients for treatment (linear), sex (linear), ambient temperature (first order, linear),  
358 and our values for spline smoothness ( $\phi$ ), with prior distributions being informed by  
359 Robertson et al (2020a). For our model intercepts, we used gamma distributed priors  
360 with  $\alpha$  values of 60 and 50 ( $T_s$  models and  $q_{Tot}$  models respectively), and  $\beta$  values of  
361 2 (both  $T_s$  models and  $q_{Tot}$  models) thus assuming positive  $T_s$  and  $q_{Tot}$  values at an  
362 ambient temperature of 0°C, with peak densities of approximately 30°C and 25 mW  
363 respectively. In all models, priors for treatment type and sex were normally distributed  
364 with means of 0 and -1 respectively, and standard deviations of 2.5, whilst those for  $\phi$   
365 were gamma distributed with  $\alpha = 2$ , and  $\beta = 0.5$  owing to low expected "wigginess"  
366 in our smooth terms. Lastly, for our first order slope of ambient temperature, we used  
367 gamma distributed priors ( $\alpha = 4$ ,  $\beta = 2$ ) in our models pertaining to  $T_s$  and normally  
368 distributed priors (mean = -5, s.d. = 5) in our models pertaining to  $q_{Tot}$  because the  
369 relationship between ambient temperature and  $T_s$  is expected to be positive, whilst  
370 that between ambient temperature and  $q_{Tot}$  is expected to be negative (Robertson *et al.*  
371 2020b). Uninformative priors were used for all other model parameters; specifically,  
372 priors for the standard deviation of population level and group level predictors followed  
373 student's t distributions with 3 degree of freedom, location parameters of 0 and a  
374 scale factors of 3.4. Similarly, priors for sigma parameters also followed student's t  
375 distributions with 3 degrees of freedom and location parameters of 0, however, scale  
376 factors were reduced to 2.5.

### 377 2.4.2 | Repeatability estimates

378 To calculate repeatability of stress-induced changes in  $T_s$  and  $q_{Tot}$ , we followed meth-  
379 ods described by Araya-Ajoy *et al.* (2015). Their methods, however, are largely de-  
380 scriptive and do not test the presence or absence of trait repeatability within exper-

imental context. To correct for this, we constructed null models (i.e. models with individual identities scrambled) for  $T_s$  and  $q_{Tot}$  across both acute and chronic time-periods, then compared mean repeatability estimates (per Markov chain iteration) acquired from true and null model posterior distributions. Here, a significant increase in repeatability values derived from true models relative to those derived from null models suggests that true repeatability values could not be explained by biases in the experimental process alone. Null models were constructed by randomly allocating individual identities to each heat transfer estimate, then re-running hierarchical models as described above (Figure 2). To control for possible effects of treatment order during identity randomisation, we limited possible identity assignments to individuals that had experienced the same treatment order as the true individual from which the  $T_s$  or  $q_{Tot}$  was obtained. Mean repeatability estimates were then compared between our true and null models using two, one-way, non-linear hypothesis tests in the R package "brms" (Bürkner, 2017). For all hypothesis tests, priors for true and null repeatability estimates were beta distributed with peaks at 0 ( $\alpha = 1$ , and  $\beta = 4$ ). Bayes factors (K), representing support for true repeatability estimates being greater than null repeatability estimates, were calculated from each hypothesis test using the Savage-Dickey density ratio method (Wagenmakers *et al.*, 2010).

### 2.4.3 | Effects of stress exposure on repeatability estimates

Traits under directional or stabilising selection are thought to display lower variability than those that are selectively neutral (e.g. Gibson & Bradley 1974; Lande & Arnold 1983; Van Homrigh *et al.* 2007; but see Kotiaho *et al.* 2001). Furthermore, the potential for traits to respond to selection is contingent upon trait expression being consistent across time (e.g. repeatable; Dochtermann *et al.* 2015; but see Dohm 2002). Thus, the presence of both high repeatability (R) and relatively low residual varia-

tion (" $\epsilon$ " in a linear or additive model) is suggestive of previous or current selection acting upon a trait's expression, if all other environmental variables and sources of measurement error are controlled (i.e wherein  $\epsilon$  is the sum of residual variation explained by external environmental factors, measurement error, and within-individual variability; suggestive in Gibson & Bradley 1974 and Boake 1989). In our experiment, both stress-exposed and control individuals experienced the same environmental conditions, and measurement error around  $T_s$  and  $q_{Tot}$  was unlikely to differ systematically between stress-exposed and control treatments. Thus, to test for evidence of enhanced stabilising or directional selection (past or current) on the expression of  $T_s$  and  $q_{Tot}$  during stress exposure relative to resting conditions, we compared error and repeatability estimates obtained for stress-exposed and control treatments across both short and long-term time-frames (e.g. acute and chronic, respectively). To do so, both error and repeatability estimates drawn from posterior distributions of acute and chronic models (pertaining to both  $T_s$  and  $q_{Tot}$ ; described above) were compared using one-way, non-linear hypothesis tests as described previously (subsection "Repeatability estimates"). Priors for repeatability and error estimates under control and stress-exposed conditions were beta ( $\alpha = 1$ ;  $\beta = 4$ ) and normally distributed (mean = 0, s.d. = 0.25) respectively. Again, Bayes factors were calculated for each test using the Savage-Dickey density ratio method (Wagenmakers *et al.*, 2010), with results representing relative support for either decreased error or increased repeatability within stress exposure treatments when compared with control treatments.

#### 2.4.4 | Effects of urbanisation on stress-induced thermal responses

To test whether flexible changes in  $T_s$  and  $q_{Tot}$  accompanying acute stress exposures differed between urban and rural chickadees, we first extracted mean coefficients for the interactions between treatment type and time post stress exposure for each individual.

<sup>431</sup> vidual from the posterior distribution of our acute models. Mean coefficients were  
<sup>432</sup> then compared between capture ecotypes using Bayesian "ANOVAs" in the R pack-  
<sup>433</sup> age "BayesFactor" (version 0.9.12.4.2; Morey *et al.* 2019) with capture location (one  
<sup>434</sup> of six) included as a group-level intercept. To test whether chronic changes in  $T_s$   
<sup>435</sup> and  $q_{Tot}$  following stress exposures differed between individuals from urban and rural  
<sup>436</sup> locations, we used a similar approach, however, mean coefficients for the interactions  
<sup>437</sup> between ambient temperature and treatment type were extracted from posterior dis-  
<sup>438</sup> tributions and used as response values. Priors for the effect of capture ecotype and  
<sup>439</sup> capture location on individual slopes were weak and Cauchy distributed with scale  
<sup>440</sup> parameters of  $2^{1/2}$  and 1 respectively, whilst Jeffreys priors were used for our intercept  
<sup>441</sup> and residual error term ( $\tau$ ) (Rouder *et al.*, 2012).

## <sup>442</sup> 3 | RESULTS

<sup>443</sup> Credible intervals (95%) are reported for model coefficients in crotchets. All reported  
<sup>444</sup> means are marginal and are given  $\pm$  one standard deviation (s.d.).

### <sup>445</sup> 3.1 | Stress-induced changes in body surface temperature and <sup>446</sup> peripheral heat loss are repeatable

<sup>447</sup> Our analyses detected rapid and pronounced changes in both eye region tempera-  
<sup>448</sup> ture (" $T_s$ ") and heat loss from the eye region (" $q_{Tot}$ ") of chickadees following stress  
<sup>449</sup> exposure " $T_s$ :  $\beta = 1.68 [0.36, 4.58]$ ;  $q_{Tot}$ :  $\beta = 2.79 [0.86, 6.90]$ "; Table 1). Similar and  
<sup>450</sup> simultaneous changes in  $T_s$  and  $q_{Tot}$  were not detected in nearby control individuals  
<sup>451</sup> (Table 1). Interestingly, the magnitude and direction of stress-induced  $T_s$  and  $q_{Tot}$   
<sup>452</sup> responses were dependent upon ambient temperature ( $T_s$ :  $\phi = 4.85 [0.62, 10.90]$ ;  $q_{Tot}$ :

<sup>453</sup>  $\phi = 6.58 [0.64, 15.50]$ ; Table 1). Specifically, at low ambient temperatures (i.e. those  
<sup>454</sup> below thermoneutrality;  $< 14^{\circ}\text{C}$ ), individuals exposed to stressors displayed rapid and  
<sup>455</sup> transient increases in  $T_s$  and  $q_{\text{Tot}}$ , with elevations in  $T_s$  and  $q_{\text{Tot}}$  persisting for approx-  
<sup>456</sup> imately 30 minutes (1800 seconds) after stressor completion (Figures 3a and 3b). At  
<sup>457</sup> our lowest observed ambient temperature ( $3^{\circ}\text{C}$ ),  $T_s$  among stress-exposed individuals  
<sup>458</sup> increased by an average of  $5.53^{\circ}\text{C} \pm 0.154^{\circ}\text{C}$  (with respect to baseline measurements)  
<sup>459</sup> immediately upon stressor completion (Figure 3a), and this increase corresponded to  
<sup>460</sup> a rise in  $q_{\text{Tot}}$  of  $11.50 \pm 0.24 \text{ mW}$  (Figure 3b). In contrast, at high ambient temper-  
<sup>461</sup> atures (i.e. those above thermoneutrality;  $> 30^{\circ}\text{C}$ ), an inverted response among stress  
<sup>462</sup> exposed individuals was detected, with individuals displaying rapid and transient re-  
<sup>463</sup> ductions in  $T_s$  and  $q_{\text{Tot}}$  (Figures 3a and 3b) in response to stress exposures (albeit small).  
<sup>464</sup> At these ambient temperatures, decreases in  $T_s$  and  $q_{\text{Tot}}$  persisted for approximately  
<sup>465</sup> 20 minutes (1200 seconds) following stressor completion, with mean  $T_s$  and  $q_{\text{Tot}}$  de-  
<sup>466</sup> creasing by approximately  $1.15^{\circ}\text{C} \pm 0.152^{\circ}\text{C}$  and  $2.23 \pm 0.24 \text{ mW}$  respectively at  
<sup>467</sup> our highest observed ambient temperature ( $38.5^{\circ}\text{C}$ ; again, with respect to baseline  
<sup>468</sup> measurements) upon stressor completion (Figures 3a and 3b). A small effect of time  
<sup>469</sup> post stress exposure on both  $T_s$  and  $q_{\text{Tot}}$  among control individuals was detected ( $\phi$   
<sup>470</sup>  $= 0.45 [0.03, 1.90]$ ; Table 1), however, neither increases nor decreases in  $T_s$  and  $q_{\text{Tot}}$   
<sup>471</sup> were detectable following onset of stress exposures (here, in the nearest-by flight en-  
<sup>472</sup> closures designated for stress exposure treatments) above or below the thermoneutral  
<sup>473</sup> zone (Figure 3b). Neither  $T_s$  nor  $q_{\text{Tot}}$  differed between sexes ( $T_s$ :  $\beta_{\text{Sex}} = -0.08 [-0.46,$   
<sup>474</sup>  $0.33]$ ;  $q_{\text{Tot}}$ :  $\beta_{\text{Sex}} = -0.17 [-0.86, 0.49]$ ; Table 1), and treatment type alone did not in-  
<sup>475</sup> fluence each measure ( $T_s$ :  $\beta_{\text{Treatment}} = 0.26 [-0.19, 0.99]$ ;  $q_{\text{Tot}}$ :  $\beta_{\text{Treatment}} = 0.29 [-0.28,$   
<sup>476</sup>  $1.25]$ ; Table 1).

<sup>477</sup> Beyond the acute responses, our analyses also detected chronic effects of stress expo-

478   sures on  $T_s$  and  $q_{Tot}$  across our sample population ( $T_s$  model:  $\beta = 1.81$ , [0.32, 5.58];  
479    $q_{Tot}$  model:  $\beta = 2.51$ , [0.61, 6.92]; Table 2). Specifically, both  $T_s$  and  $q_{Tot}$  of stress-  
480   exposed individuals decreased at low ambient temperatures and increased at high am-  
481   bient temperatures relative to controls (Table 2; Figure 4). On average,  $T_s$  was  $1.89^\circ\text{C}$   
482    $\pm 1.22^\circ\text{C}$  lower in stress-exposed individuals than control individuals at our lowest  
483   observed ambient temperature, and  $1.64^\circ\text{C} \pm 0.95^\circ\text{C}$  higher in stress-exposed indi-  
484   viduals than control individuals at our highest observed ambient temperature. Such  
485   trends in  $T_s$  corresponded to reductions in  $q_{Tot}$  of approximately  $3.75 \pm 2.56 \text{ mW}$   
486   at our lowest observed ambient temperature, and increases in  $q_{Tot}$  of approximately  
487    $2.56 \pm 1.99 \text{ mW}$  at our highest observed ambient temperature among stress exposed  
488   individuals relative to controls (Figure 4). Similar to our results pertaining to acute  
489   thermal responses, neither  $T_s$  nor  $q_{Tot}$  differed between sexes in our chronic model  
490   ( $T_s$ :  $\beta_{Sex} = 0.02$  [-0.41, 0.44];  $q_{Tot}$  model:  $\beta_{Sex} = 0.03$  [-0.71, 0.76]; Table 2) and no  
491   effect of treatment alone on  $T_s$  or  $q_{Tot}$  was detected ( $T_s$ :  $\beta_{Treatment} = 0.02$  [-0.16, 0.20];  
492    $q_{Tot}$ :  $\beta_{Treatment} = 0.00$  [-0.29, 0.29]).

493   As predicted, acute stress-induced changes in  $T_s$  and  $q_{Tot}$  were significantly repeat-  
494   able among chickadees. Namely, repeatability values calculated from our true models  
495   exceeded those calculated from our null model (i.e. with individual identities scram-  
496   bled; non-linear hypothesis test:  $K_{T_s} > 100$ ;  $K_{q_{Tot}} = 47.00$ ; Figure 5a and SFigure  
497   5a), suggesting that repeatability of acute thermal responses to stress exposure not  
498   only exceeded zero, but also could not be explained by biases in our experimental  
499   methodology. Nevertheless, the degree to which these acute thermal responses were  
500   repeatable among chickadees was low (surface temperature [ $T_s$ ]:  $R_{\text{stress exposure}} = 0.14$   
501   [0.03, 0.32]; heat transfer [ $q_{Tot}$ ]:  $R_{\text{stress exposure}} = 0.11$  [0.02, 0.27]; Table 1), suggesting  
502   that whilst some variation in acute thermal responses is probably attributable to con-

503 sistent differences in stress-responsive phenotypes among individuals, the majority of  
504 such variation is perhaps better explained by other sources of variation (e.g. environ-  
505 mental or measurement). Similar to acute changes in  $T_s$  and  $q_{Tot}$ , chronic changes in  
506  $T_s$  and  $q_{Tot}$  following stress exposure (or "chronic reaction norms") were significantly  
507 repeatable among chickadees. Again, repeatability values estimated from our true  
508 models exceeded those estimated from our null models, suggesting that repeatability  
509 of chronic changes in  $T_s$  and  $q_{Tot}$  observed in our study were unlikely to be explained  
510 by biases in our experimental method (non-linear hypothesis tests comparing true and  
511 null models;  $K > 100$  for both  $T_s$  and  $q_{Tot}$ ; Figure 5b and SFigure 5b). Here, however,  
512 repeatability of chronic reaction norms among chickadees was high ( $R_{T_s} = 0.61$  [0.35,  
513 0.81];  $R_{q_{Tot}} = 0.67$  [0.44, 0.84]; Table 2), indicating that stress-induced changes in  $T_s$   
514 and  $q_{Tot}$  consistently varied among individuals.

### 515 3.2 | Evidence for stabilising or directional selection on stress- 516 induced changes in body surface temperature and periph- 517 eral heat loss

518 Among stress-exposed individuals,  $T_s$  of control individuals was significantly more  
519 variable and less consistent across acute time periods (i.e.  $\leq 1$  hour) than rested indi-  
520 viduals, after controlling for circadian rhythms and environmental effects (e.g. ambi-  
521 ent temperature, solar radiation;  $\sigma_{Control} = 1.21$  [1.19, 1.24],  $\sigma_{Stress} = 1.18$  [1.14, 1.22];  
522  $R_{control} = 0.07$  [0.01, 0.18],  $R_{stress\ exposure} = 0.14$  [0.03, 0.32]; Table 1). As predicted,  
523 these difference in variance and repeatability between treatments were strongly and  
524 moderately supported by non-linear hypothesis tests respectively ( $K_{variance} = 72.47$ ;  
525  $K_{repeatability} = 6.66$ ; SFigure 6). Similarly,  $q_{Tot}$  at the eye region of chickadees was  
526 both slightly less variable and more repeatable during stress exposure treatments than  
527 control treatments ( $\sigma_{Control} = 2.09$  [2.06, 2.13],  $\sigma_{Stress} = 2.06$  [1.99, 2.12];  $R_{control} = 0.06$

528 [0.01, 0.16],  $R_{\text{stress exposure}} = 0.11$  [0.02, 0.27]; Table 1). These differences in unexplained  
529 variability and repeatability, however, were only moderately and weakly supported  
530 by non-linear hypothesis tests respectively ( $K_{\text{variance}} = 10.65$ ;  $K_{\text{repeatability}} = 5.24$ ; SFigure  
531 7).

532 Similar to acute time periods,  $T_s$  of chickadees was more variable and less repeatable in  
533 control treatments than in stress exposure treatments across chronic time periods (i.e.  
534  $\leq 30$  days), after controlling for circadian and environmental effects ( $\sigma_{\text{Control}} = 1.20$   
535 [1.18, 1.23],  $\sigma_{\text{Stress}} = 1.17$  [1.13, 1.21];  $R_{\text{control}} = 0.34$  [0.17, 0.56],  $R_{\text{stress exposure}} = 0.61$   
536 [0.35, 0.81]; Table 2). Again, as predicted, these differences in variance and repeata-  
537 bility were strongly and moderately supported by respective non-linear hypothesis  
538 tests ( $K_{\text{variance}} = 48.32$ ;  $K_{\text{repeatability}} = 15.51$ ; SFigure 8). Variability and repeatability  
539 of  $q_{\text{Tot}}$  across chronic time periods followed similar patterns, with variability again  
540 being lower and repeatability again being higher in stress-exposed chickadees, when  
541 compared with rested (i.e. control) chickadees ( $\sigma_{\text{Control}} = 2.07$  [2.04, 2.11],  $\sigma_{\text{Stress}} =$   
542 2.04 [1.98, 2.10];  $R_{\text{control}} = 0.41$  [0.22, 0.63],  $R_{\text{stress exposure}} = 0.67$  [0.44, 0.84]; Table 2).  
543 These differences were moderately and strongly supported by non-linear hypothesis  
544 tests respectively ( $K_{\text{variance}} = 9.62$ ;  $K_{\text{repeatability}} = 16.73$ ; SFigure 9), as predicted.

### 545 3.3 | Stress-induced thermal responses do not differ between 546 urban and rural individuals

547 Interestingly, the magnitude of acute changes in  $T_s$  or  $q_{\text{Tot}}$  (or "acute reaction norms")  
548 following stress exposure did not differ between chickadees captured from urban or  
549 rural ecotypes ( $T_s$ :  $\mu_{1:\hat{urban}} = 0.09$  [-0.25, 0.82];  $\mu_{1:\hat{rural}} = 0.14$  [-0.23, 0.54];  $q_{\text{Tot}}$ :  
550  $\mu_{1:\hat{urban}} = 0.19$  [-0.35, 0.87];  $\mu_{1:\hat{rural}} = 0.14$  [-0.34, 1.28]; Figure 6; SFigure 10). Indeed,

<sup>551</sup> ANOVAs including capture ecotype as a population-level predictor were less likely to  
<sup>552</sup> explain the magnitude of  $T_s$  or  $q_{Tot}$  responses among individuals than ANOVAs with-  
<sup>553</sup> out ( $T_s$ :  $K = 0.25$ ;  $q_{Tot}$ :  $K = 0.24$ ). Similar results were detected at the chronic level,  
<sup>554</sup> with the magnitude of chronic stress-induced changes in  $T_s$  and  $q_{Tot}$  (or, "chronic  
<sup>555</sup> reaction norms") remaining similar between urban- and rural-origin chickadees ( $T_s$ :  
<sup>556</sup>  $\mu_{1:\hat{urban}} = -0.25 [-4.20, 1.88]$ ;  $\mu_{1:\hat{rural}}$ ;  $q_{Tot}$ :  $\mu_{1:\hat{urban}} = -0.25 [-4.20, 1.88]$ ;  $\mu_{1:\hat{rural}} = 0.20$   
<sup>557</sup>  $[-3.68, 5.89$ ; Figure 6). Again, ANOVAs including capture ecotype as a predictor were  
<sup>558</sup> less likely to explain the magnitude of chronic changes in  $T_s$  and  $q_{Tot}$  than ANOVAs  
<sup>559</sup> without ( $T_s$ :  $0.24$ ;  $q_{Tot}$ :  $0.25$ ).

## <sup>560</sup> 4 | DISCUSSION

### <sup>561</sup> 4.1 | Acute and chronic thermal responses to stress exposure are <sup>562</sup> repeatable

<sup>563</sup> Our results show that flexible changes in surface temperature ( $T_s$ ) and rate of heat  
<sup>564</sup> transfer ( $q_{Tot}$ ) following stress exposures are repeatable in chickadees, whether ob-  
<sup>565</sup> served across acute or protracted (i.e. chronic) time periods. Such repeatability fulfills  
<sup>566</sup> a critical first prediction of the hypothesis that stress-induced flexibility of  $T_s$  and  $q_{Tot}$   
<sup>567</sup> may experience evolutionary responses to selection. Notably, however, the extent to  
<sup>568</sup> which flexibility of  $T_s$  and  $q_{Tot}$  was repeatable appeared to depend upon the time pe-  
<sup>569</sup> riod of observation (Figure 5 and SFigure 5). Across acute time periods, the shape and  
<sup>570</sup> magnitude of stress-induced  $T_s$  and  $q_{Tot}$  responses were appreciably similar among  
<sup>571</sup> individuals (Figures 3b and 5a; SFigure 5a). Across chronic time-periods, however, a  
<sup>572</sup> considerably wider range of stress-responsive phenotypes among individuals emerged  
<sup>573</sup> (Figures 4 and 5b; SFigure 5b). To our knowledge, our study is the first to report re-  
<sup>574</sup> peatability of stress-induced flexibility of  $T_s$  and  $q_{Tot}$  in any vertebrate.

575 The high degree with which chronic responses to stress exposure varied among our  
576 study individuals highlights that, despite a clear average trend among individuals (Figure  
577 4; Table 1), reductions in average  $T_s$  and  $q_{Tot}$  in the cold and increases in average  
578  $T_s$  and  $q_{Tot}$  in the warmth are clearly not generalisable responses to repeated stress  
579 perception in birds. Among some individuals, for example, repeated stress exposure  
580 appeared to elicit the reverse response, with mean  $T_s$  and  $q_{Tot}$  rising in the cold and  
581 decreasing in the heat (Figure 4). If the emergence of such chronic stress-induced re-  
582 sponses are largely fixed within individuals, as our study suggests, theorised energetic  
583 benefits ascribed to this response (e.g. Robertson *et al.* 2020b) may only be accrued by  
584 some and not all individuals. Given that survivorship has been linked to efficiency of  
585 energy use in extreme and challenging environments (Parsons, 2005), such discrep-  
586 ancies in theorised energetic savings could provide opportunities for selection to act  
587 upon chronic thermal responses to stress exposure in our study species.

588 Any evolutionary responses to selection on flexibility of  $T_s$  and  $q_{Tot}$  in response to  
589 chronic stress exposures requires that this trait is underpinned by heritable genetic  
590 architecture. In this study, we chose to monitor changes in  $T_s$  and peripheral  $q_{Tot}$  in  
591 response to stress exposure alone. Therefore, whether chronic responses observed here  
592 emerge as a consequence of stress-induced changes in core body temperature, periph-  
593 eral temperature (e.g. by changes in vascular flow; Oka *et al.* 2001), or both remains  
594 unknown. Regardless of their anatomic origin, the possibility of individual differences  
595 in chronic responses arising from differences in genetic architecture is well supported.  
596 At the level of core tissues, for example, both heterothermy and facultative hypother-  
597 mia appear phylogenetically constrained (Boyles *et al.*, 2013; Gerson *et al.*, 2019), and  
598 recent studies in poultry have provided strong evidence for the direct influence of ge-

599 netic polymorphisms and differential gene transcription on heat dissipation capacity  
600 and the magnitude of core body temperature increases in supra-thermoneutral am-  
601 bient temperatures (Srikanth *et al.*, 2019; Zhuang *et al.*, 2019). Similarly, at the level  
602 of the periphery, studies in humans have elucidated several genetic polymorphisms  
603 that appear to dictate the duration and magnitude of peripheral vascular responses to  
604 cold and psychological stress (e.g. Rao *et al.* 2008; Chen *et al.* 2010; Kelsey *et al.* 2010,  
605 2012; Huang *et al.* 2012) that could have meaningful consequences on environmental  
606 heat transfer; many such polymorphisms correspond to genes with conserved func-  
607 tions among tetrapods (Vincent *et al.* 1998; Yamamoto & Vernier 2011; Céspedes *et al.*  
608 2017; Dopamine  $\beta$ -hydroxylase in sauropsids: Lovell *et al.* 2015). Consequently, vari-  
609 ation in stress-induced changes in  $T_s$  and  $q_{Tot}$  among our Chickadees may well be  
610 heritable, regardless of whether such responses are driven by changes in thermogen-  
611 esis at the core, or by changes in peripheral vascular flow and consequential changes  
612 in environmental heat transfer (Robertson *et al.* 2020a).

613 Still, we cannot refute the possibility that our observed chronic responses to stress  
614 exposure are broadly labile within individuals and dictated by energetic or resource  
615 constraints that were not measured here. For example, Robertson et al (2020b) re-  
616 cently argued that stress-induced changes in  $T_s$  and  $q_{Tot}$  may be understood as trade-  
617 offs that are predominantly manifested under negative energetic balance. It is possible  
618 that our experimental conditions may have contributed to fixed and non-random re-  
619 source allocation among individuals (e.g. via dominance interactions; Ratcliffe *et al.*  
620 2007) that dictated how stress-induced thermal responses at the eye region emerged.  
621 In such a case, any evolutionary responses to selection on this responses may better  
622 reflect patterns of resource monitoring and allocation during a challenge, rather than  
623 fixed reflexes within individuals. Experiments seeking to tease apart the influence of

624 resource availability and fixed individual variation on chronic thermal responses to  
625 stress exposure are therefore warranted.

626 To our surprise, the degree to which individuals acutely shifted their  $T_s$  and  $q_{Tot}$  in  
627 response to stress exposures displayed considerable overlap (Figures 3a and 3b). Such  
628 overlap among individuals, coupled with the significant predictive effects of other en-  
629 vironmental parameters (e.g. ambient temperature and time of day; Table 1) implies  
630 that, unlike chronic thermal responses, the manifestation of acute thermal responses  
631 to stress exposure is perhaps better explained by the combination of common trait  
632 expression and environmental effects than variation in intrinsic factors among indi-  
633 viduals. In domestic rats (*Rattus norvegicus domestica*), ambient temperature has been  
634 shown to strongly influence the magnitude of acute changes in core body tempera-  
635 ture, with responses typically being largest at low ambient temperature and smallest  
636 at high ambient temperatures (Briese 1992; reviewed in Oka 2018). Similarly, in  
637 Svalbard rock ptarmigans (*Lagopus muta hyperborea*), the magnitude of stress-induced  
638 changes in skin temperature are reportedly larger at low ambient temperature than  
639 at comparatively higher ambient temperatures (Nord & Folkow, 2019). As such, the  
640 emergence of acute, stress-induced changes in  $T_s$  and  $q_{Tot}$  in our sample population  
641 may have been largely dictated by modulatory effects of ambient temperature alone,  
642 with little remaining variation explained by phenotypic differences among individ-  
643 uals. In any case, the relatively low repeatability of acute stress-induced thermal re-  
644 sponds (observed here) highlights that the potential for this response to respond to  
645 selection in black-capped chickadees is probably low.

646 4.2 | Variation in eye region temperature and heat loss is re-  
647 duced during stress exposure

648 Interestingly, unexplained variation in both  $T_s$  and  $q_{Tot}$  was higher during control  
649 treatments than during stress exposure treatments (Tables 1–2). Additionally,  
650 both  $T_s$  and  $q_{Tot}$  were more repeatable during stress exposure treatments than  
651 control treatments (SFigures 6–9) regardless of the time period of observation (i.e.  $\leq$   
652 1 hour, or  $\leq$  30 days). Together, these trends indicate that either: 1)  $T_s$  and  $q_{Tot}$  are  
653 more tightly regulated during stress exposures than during resting conditions, or 2)  
654  $T_s$  regulation is relaxed during stress exposures, thereby allowing  $T_s$  to conform to  
655 ambient temperatures (as observed in other avian species; reviewed in Angilletta *et al.*  
656 2019). Regardless of the mechanism, the relative consistency with which  $T_s$  and  $q_{Tot}$   
657 emerge during stress exposures suggests that their maintenance has, perhaps, experi-  
658 enced stronger directional or stabilising selection than that during rested (i.e. control)  
659 conditions (our second prediction; e.g. Gibson & Bradley 1974; Lande & Arnold 1983;  
660 Van Homrigh *et al.* 2007; but see Kotiaho *et al.* 2001). Such findings lend credence to a  
661 critical role of heat-transfer regulation during stress exposure, that, to our knowledge,  
662 has received little to no research attention.

663 When contextualised with variability within other stress-physiological processes, re-  
664 duced variability of  $T_s$  and  $q_{Tot}$  during stress perception is perhaps not unusual. Vari-  
665 ability in heart rate is widely known to fall during stress exposure in many verte-  
666 brate species (e.g. Visser *et al.* 2002; Von Borell *et al.* 2007; Cyr *et al.* 2009). Simi-  
667 larly, within-individual variation in stress-induced glucocorticoid production has been  
668 reported to be lower than that of baseline production in both avian and amphibian  
669 species (e.g. Cockrem & Silverin 2002; Rensel & Schoech 2011; Narayan *et al.* 2012;

670 Grace & Anderson 2014; but see Narayan *et al.* 2013; Baugh *et al.* 2014; Lendvai *et al.*  
671 2015). Such trends indicate that the collective traits enabling individuals to conform  
672 or cope with environmental challenges (together, the "stress phenotype") have expe-  
673 rienced strong directional or stabilising selection (Ellis *et al.*, 2006). Modulation of  $T_s$   
674 and  $q_{Tot}$  during stress exposure (whether by a reduction or increase) may, therefore,  
675 simply represent a little-discussed constituent of the vertebrate stress phenotype that  
676 contributes to successful coping. Although the ultimate value of stress-induced  $T_s$  and  
677  $q_{Tot}$  modulation is unclear, the bivalent nature, ambient-temperature dependence, and  
678 direct implications on energetic savings in our study (albeit small; Figure 4) triangu-  
679 late on a relaxation of expenditure toward thermoregulation (the Thermoprotective  
680 Hypothesis; Robertson *et al.* 2020a). On the other hand, rapid increases in  $T_s$  and  $q_{Tot}$   
681 at low ambient temperatures, and rapid declines in  $T_s$  and  $q_{Tot}$  following stress expo-  
682 sure (as observed here; Figures 3a and 3b) may suggest that at the acute level, changes  
683 in  $T_s$  occur to promote enzymatic, neuronal, or muscular function during the stress  
684 responses (i.e. owing to Q10 effects: e.g. Carr & Lima 2013), rather than to reduce  
685 thermoregulatory expenses.

#### 686 4.3 | Urban and rural individual do not differ in stress-induced 687 thermal responses

688 In sharp contrast to our predictions, the degree to which  $T_s$  and  $q_{Tot}$  flexibly re-  
689 sponded to acute or chronic stress exposure did not differ between chickadees cap-  
690 tured from urban and rural environments (Figure 6 and SFigure 10). According to  
691 our results, individuals from urban environments appear no more able to flexibly shift  
692 their  $T_s$  and thermoregulatory expenditure during stress exposure than those from  
693 rural environments. We propose three possible explanations for these findings. First,  
694 insufficient generations spent within a given ecotype may have limited opportunities

for evolutionary responses to selection on stress-induced thermal responses to occur in our study species. The combination of low juvenile dispersal, high site fidelity among adults (Weise & Meyer, 1979), and relatively short generation time in our study species, however, suggests that this is unlikely (reviewed in McDonnell & Hahs 2015). Furthermore, genetic differentiation between individuals captured in urban and rural environments has recently been reported for a closely related Parid species (the great tit, *Parus major*; Perrier *et al.* 2018), supporting the possibility of responses to selection imposed by urban environments. A second, and arguably more likely explanation for our findings is that costs of urban living in chickadees are no higher than those of rural living, despite a theoretically increased frequency in stress exposure events. Although direct comparative field studies are lacking (Sepp *et al.*, 2018), trends in basal metabolic rate of another temperature bird species (the house finch, *Haemorhous mexicanus*) do suggest that energetic expenditure may not differ between individuals captured from urban and rural environments (at least, at rest: Hutton *et al.* 2018). In chickadees, urban environments may afford opportunities to access novel and abundant food sources (Robb *et al.*, 2008; Prasher *et al.*, 2019) that could offset energetic costs associated with frequent activation of emergency pathways (but see Demeyrier *et al.* 2017). Strategies to relax expenditure towards other biological process (e.g. thermoregulation), therefore, may be no more likely to emerge in urban population than rural populations. Lastly, neither acute nor chronic changes in  $T_s$  and  $q_{Tot}$  that accompany stress exposures may be heritable in chickadees. Previous studies, both within and across species, have suggested that changes in core body temperature and peripheral vascular flow during a challenge are underpinned by heritable genetic architecture (discussed above). Nevertheless, it is indeed possible that thermal responses to stress exposure at either the acute or chronic level are merely contingent upon environmental context (e.g. resource availability) and the maximum degree to

<sup>721</sup> which  $T_s$  and  $q_{Tot}$  can flexibly respond to stress exposure is fixed among individuals.  
<sup>722</sup> Further studies questioning the heritability of stress-induced thermal responses in this  
<sup>723</sup> species are, therefore, critical to understanding whether this response may provide  
<sup>724</sup> opportunities to adapt to a warming and urbanising world.

## <sup>725</sup> 4.4 | Summary

<sup>726</sup> Recent empirical studies have argued that endotherms may balance costs associated  
<sup>727</sup> with responding to perceived stressors by flexibly decreasing their  $T_s$  and  $q_{Tot}$  in the  
<sup>728</sup> cold, and flexibly increasing their  $T_s$  and  $q_{Tot}$  in warmth. By doing so, energy may  
<sup>729</sup> be allocated away from costly thermogenesis or evaporative cooling, and toward the  
<sup>730</sup> immediate demands of coping with the challenge at hand (Robertson *et al.* 2020a). In  
<sup>731</sup> chickadees, we tested whether such stress-induced flexibilities of  $T_s$  and  $q_{Tot}$  are re-  
<sup>732</sup> peatable among individuals and thus offer opportunities for endotherms to cope with  
<sup>733</sup> costs that typify urbanised environments, across generations. As predicted, we show  
<sup>734</sup> that both acute and chronic changes in  $T_s$  and  $q_{Tot}$  during stress exposure are repeat-  
<sup>735</sup> able, however, only those at the chronic level displayed meaningfully high repeatabil-  
<sup>736</sup> ity estimates ( $T_s$ :  $R_{chronic} = 0.61$ ;  $q_{Tot}$ :  $R_{chronic} = 0.67$ ). Furthermore, we show that both  
<sup>737</sup>  $T_s$  and  $q_{Tot}$  are less variable within individuals, and more variable among individuals  
<sup>738</sup> during experimental stress exposure than during control treatment, suggesting that  
<sup>739</sup> regulation of  $T_s$  and  $q_{Tot}$  during the stress response has probably experienced direc-  
<sup>740</sup> tional or stabilising selection. Both trends, to our knowledge, are yet to be reported  
<sup>741</sup> in any vertebrate. To our surprise, neither acute, nor chronic flexibility of  $T_s$  and  $q_{Tot}$   
<sup>742</sup> in response to stress exposure differed between urban- and rural-origin chickadees.  
<sup>743</sup> Together, our results suggest that whilst flexibility of  $T_s$  and  $q_{Tot}$  meet a critical first  
<sup>744</sup> criterion for responsiveness to selection and may enhance energetic efficiency of some

<sup>745</sup> but not all individuals, those residing in urban environments are no more likely to  
<sup>746</sup> acquire benefits associated with this flexibility than those in rural environments.

## Tables

TABLE 1 Acute effects of stress exposure on eye region temperature ( $T_s$ ) and dry heat transfer ( $q_{Tot}$ ) of black-capped chickadees ( $n = 19$ ;  $n = 9$  females,  $n = 10$  males); results of two hierarchical GAMMs. Obelisks ( $\dagger$ ) represent smooth terms, for which estimates refer to the degree of smoothness ( $\phi$ : 0 = linear slope). Estimates for remaining population-level terms represent linear slopes, whilst those for group-level effects represent standard deviations. Degree of smoothness and 95% credible intervals ("CIs") for tensor products represent means across penalisation groupings, and effective sample sizes represent sums across groupings. Eye region temperature measurements were estimated from infrared thermographic images ( $n = 5599$ ) captured across 60 days.  $T_s$  model:  $R^2 = 0.85$ ;  $q_{Tot}$  model:  $R^2 = 0.94$ . Asterisks (\*) represent statistically significant terms (95% credible intervals do not cross zero).

Population-level Predictors			
Term	T <sub>s</sub> Estimate [95% CIs]	q <sub>Tot</sub> Estimate [95% CIs]	Effective Sample Size (T <sub>s</sub> /q <sub>Tot</sub> )
Intercept*	33.09 [30.84, 35.10]	19.02 [15.16, 25.30]	3644/3600
Treatment	0.26 [-0.19, 0.99]	0.29 [-0.28, 1.25]	3726/3917
Sex (Male)	-0.08 [-0.46, 0.33]	-0.17 [-0.86, 0.49]	3536/3714
†Ambient Temperature*	1.63 [0.38, 5.03]	1.38 [0.16, 5.00]	3537/3680
†Ambient Temperature: Treatment*	1.47 [0.19, 5.07]	1.87 [0.29, 5.74]	2870/3191
†Time Post Stress Exposure*	0.45 [0.03, 1.90]	0.65 [0.05, 2.52]	3273/3440
†Time Post Stress Exposure: Treatment*	1.68 [0.36, 4.58]	2.79 [0.86, 6.90]	3566/3679
†[Time Post Stress Exposure $\otimes$ Ambient Temperature]: Treatment*	4.85 [0.62, 10.90]	6.58 [0.64, 15.50]	10141/10674
†Hour $\otimes$ Orientation*	3.68 [0.65, 10.10]	4.19 [0.64, 10.70]	10571/10674
Group-level Predictors			
Bird Identity	0.32 [0.20, 0.50]	0.56 [0.35, 0.87]	3763/3121
Date of Photo	1.79 [1.37, 2.32]	3.30 [2.50, 4.20]	3254/3101
Flight Enclosure Identity	1.51 [0.34, 4.19]	5.06 [0.96, 14.19]	3658/3486
Bird Identity: Time Post Stress Exposure (Control)	0.36 [0.11, 0.56]	0.49 [0.14, 0.90]	3397/3496
Bird Identity: Time Post Stress Exposure (Stress Exposed)	0.46 [0.21, 0.81]	0.71 [0.28, 1.26]	3459/3390
Residual Variance and Repeatability			
$\sigma_{\text{Control}}$	1.21 [1.19, 1.24]	2.09 [2.06, 2.13]	3420/3917
$\sigma_{\text{Stress exposure}}$	1.18 [1.14, 1.22]	2.06 [1.99, 2.12]	3542/3679
R <sub>Control</sub>	0.07 [0.01, 0.18]	0.06 [0.01, 0.16]	3420/3917
R <sub>Stress exposure</sub>	0.14 [0.03, 0.32]	0.11 [0.02, 0.27]	3542/3679

TABLE 2 Chronic effects of stress exposure on eye region temperature ( $T_s$ ) and dry heat transfer ( $q_{Tot}$ ) of black-capped chickadees across ambient temperature ( $n = 19$ ;  $n = 9$  females,  $n = 10$  males); results of a hierarchical, Bayesian GAMMs. Results for each level of tensor products are separated by semicolons. Obelisks ( $\dagger$ ) represent smooth terms, for which estimates refer to the degree of smoothness ( $\phi$ : 0 = linear slope). Estimates for remaining population-level terms represent linear slopes, whilst those for group-level effects represent standard deviation explained by respective terms. Again, degree of smoothness and 95% credible intervals ("CIs") for tensor products represent means across penalisation groupings, and effective sample sizes represent sums across groupings. Eye region temperature measurements were estimated from infrared thermographic images ( $n = 5832$ ) captured across 60 days.  $T_s$  model:  $R^2 = 0.85$ ;  $q_{Tot}$  model:  $R^2 = 0.94$ . Asterisks (\*) represent statistically significant terms (95% credible intervals do not cross zero).

Population-level Predictors			
Term	T <sub>s</sub> Estimate [95% CIs]	q <sub>Tot</sub> [95% CIs]	Effective Sample Size (T <sub>s</sub> /q <sub>Tot</sub> )
Intercept*	32.90 [30.73, 34.75]	18.68 [14.87, 25.23]	3479/3419
Treatment	0.02 [-0.16, 0.20]	0.00 [-0.29, 0.29]	3628/3370
Sex (Male)	0.02 [-0.41, 0.44]	0.03 [-0.71, 0.76]	3387/3628
†Ambient Temperature*	1.57 [0.31, 5.31]	1.28 [0.12, 4.76]	3742/3425
†Ambient Temperature: Treatment*	1.81 [0.32, 5.58]	2.51 [0.61, 6.92]	3608/3299
†Hour $\otimes$ Orientation*	3.41 [0.72, 8.48]	4.17 [0.81, 9.51]	7206/6862
Group-level Predictors			
Bird Identity	0.35 [0.23, 0.57]	0.62 [0.40, 1.00]	3551/3263
Date of Photo	1.83 [1.40, 2.36]	3.31 [2.56, 4.26]	3598/3470
Flight Enclosure Identity	1.50 [0.31, 4.36]	4.85 [0.81, 13.86]	3467/3508
Bird Identity: Ambient Temperature (Control)	0.88 [0.54, 1.36]	1.76 [1.09, 2.74]	3633/3507
Bird Identity: Ambient Temperature (Stress exposure)	1.52 [0.85, 2.42]	3.05 [1.82, 4.74]	3608/3458
Residual Variance and Repeatability			
$\sigma_{\text{Control}}$	1.20 [1.18, 1.23]	2.07 [2.04, 2.11]	3503/3846
$\sigma_{\text{Stress exposure}}$	1.17 [1.13, 1.21]	2.04 [1.98, 2.10]	3297/3461
R <sub>Control</sub>	0.34 [0.17, 0.56]	0.41 [0.22, 0.63]	3503/3846
R <sub>Stress exposure</sub>	0.61 [0.35, 0.81]	0.67 [0.44, 0.84]	3297/3461

## Figures



FIGURE 1 Depiction of experimental stress exposure (novel object) and infrared thermographic imaging in a selected flight enclosure. Black-capped chickadees ( $n = 5$ ) within a given flight enclosure were simultaneously exposed to each individual stressor (here, the presence of a garden gnome), whilst individuals at raised feeding platforms were passively imaged with a remotely activated infrared thermographic camera.

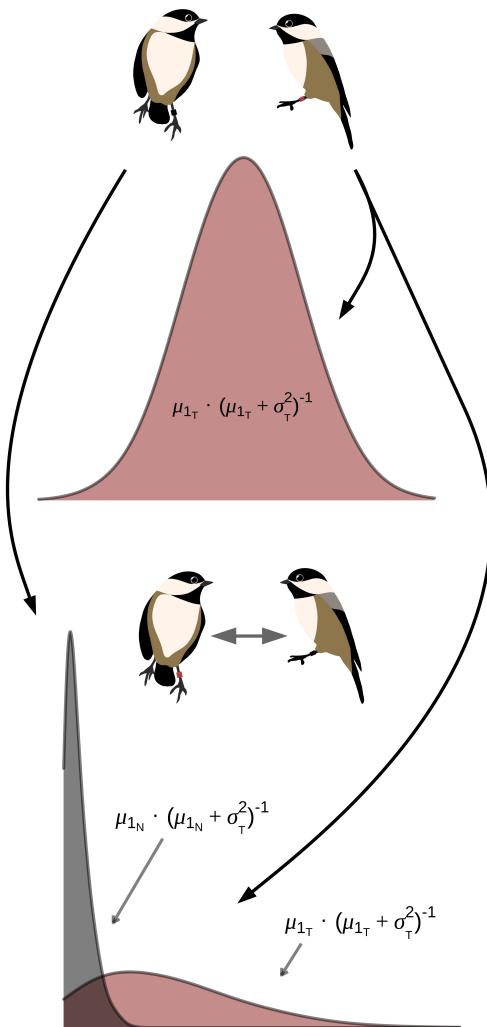


FIGURE 2 Method used to test for repeatability of stress-induced thermal responses among black-capped chickadees, whilst controlling for possible biases in the experimental process. Repeatability values were calculated from a true model (maroon; subscripted "T") using methods described by Araya-Ajoy et al (2015). Individual identities were then scrambled to produce a null model (grey; subscripted "N"), from which repeatability values were again calculated as described above. Final repeatability estimates from true and null models were compared statistically.

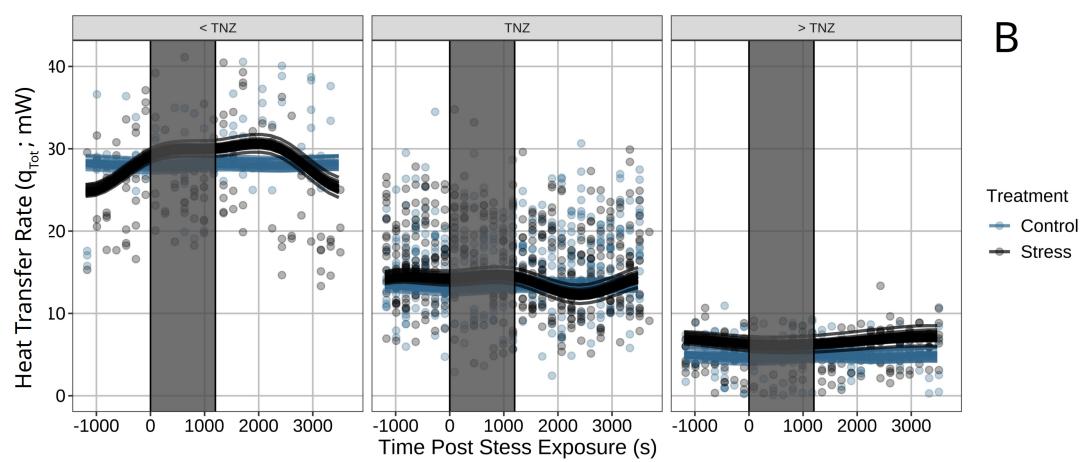
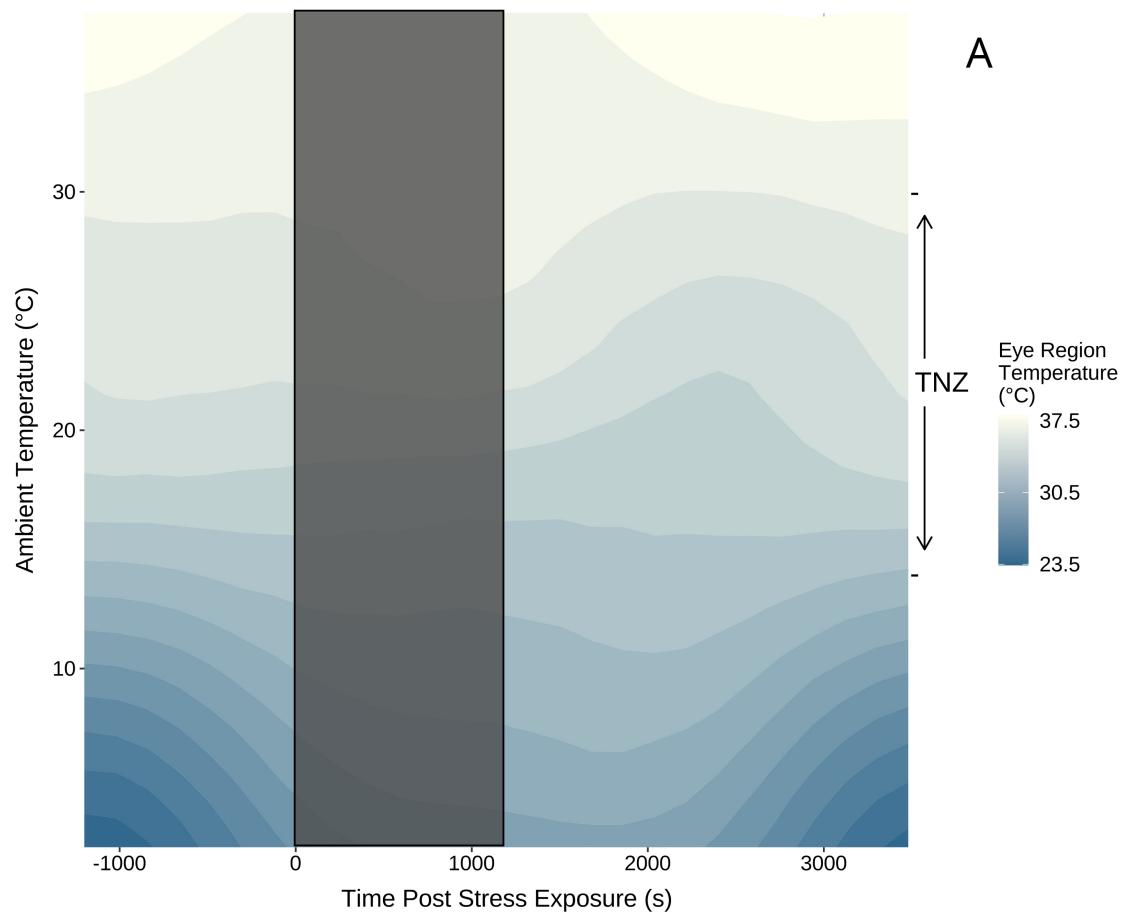


FIGURE 3 Acute changes in eye region temperature ( $T_s$ ) and dry heat transfer ( $q_{Tot}$ ) following stress exposure in black-capped chickadee ( $n = 19$ ) across ambient temperature. A | Average change in  $T_s$  following stress exposure across ambient temperature ( $^{\circ}\text{C}$ ) and time since exposure (s). Averages are derived from a Bayesian generalised additive mixed effects model (GAMM) and are marginalised across all other model predictors.  $T_s$  decreases after stress exposure at ambient temperatures below thermoneutrality, and increases after stress exposure at ambient temperatures above thermoneutrality. B | Changes in  $q_{Tot}$  of black-capped chickadees across both control and stress-exposed treatments, where slopes per treatment are permitted to vary among individuals. Each line represents the trend for a given individual at temperatures below, within, and above the thermoneutral zone (TNZ; estimated from Grossman and West, 1977), as predicted from a Bayesian GAMM. Dots represent averages per individual across 3 minutes of observation. Both trend lines and dots represent averages for each ambient temperature grouping (< TNZ, TNZ, > TNZ). Grey rectangles in panels A and B represent time when stress exposure treatments were applied in stress-exposed treatment groups.  $T_s$  and  $q_{Tot}$  were estimated by infra-red thermography ( $n = 5832$  images) across 60 days.

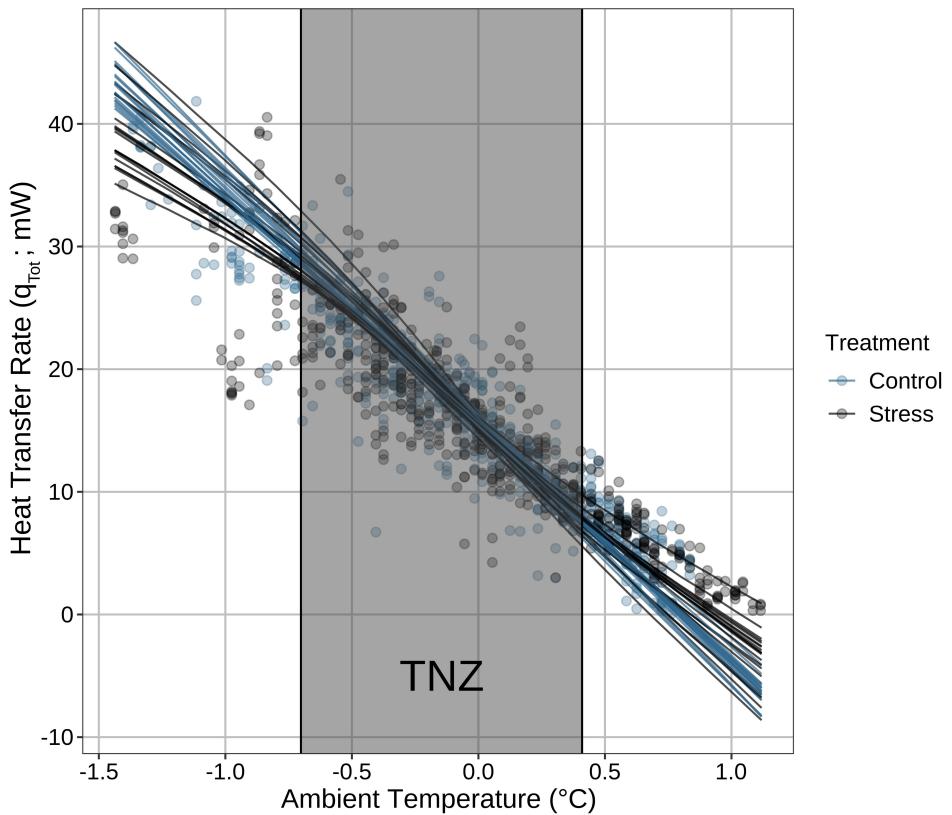


FIGURE 4 Chronic changes in dry heat transfer ( $q_{\text{Tot}}$ ) at the eye region of black-capped chickadees ( $n = 19$ ) following stress exposure across varying ambient temperatures. Individual lines represents the predicted correlation between ambient temperature (here, mean-centered) and  $q_{\text{Tot}}$  of individual black-capped chickadees during stress-exposure or control treatments. Grey rectangle represents the thermoneutral zone (TNZ) for black-capped chickadees (estimated from Grossman and West, 1977). Correlations are estimated from a Bayesian generalised additive mixed effects model (GAMM) and marginalised across all environmental and experimental parameters.  $q_{\text{Tot}}$  values were estimated by infra-red thermography ( $n = 5832$  images) across 60 days.

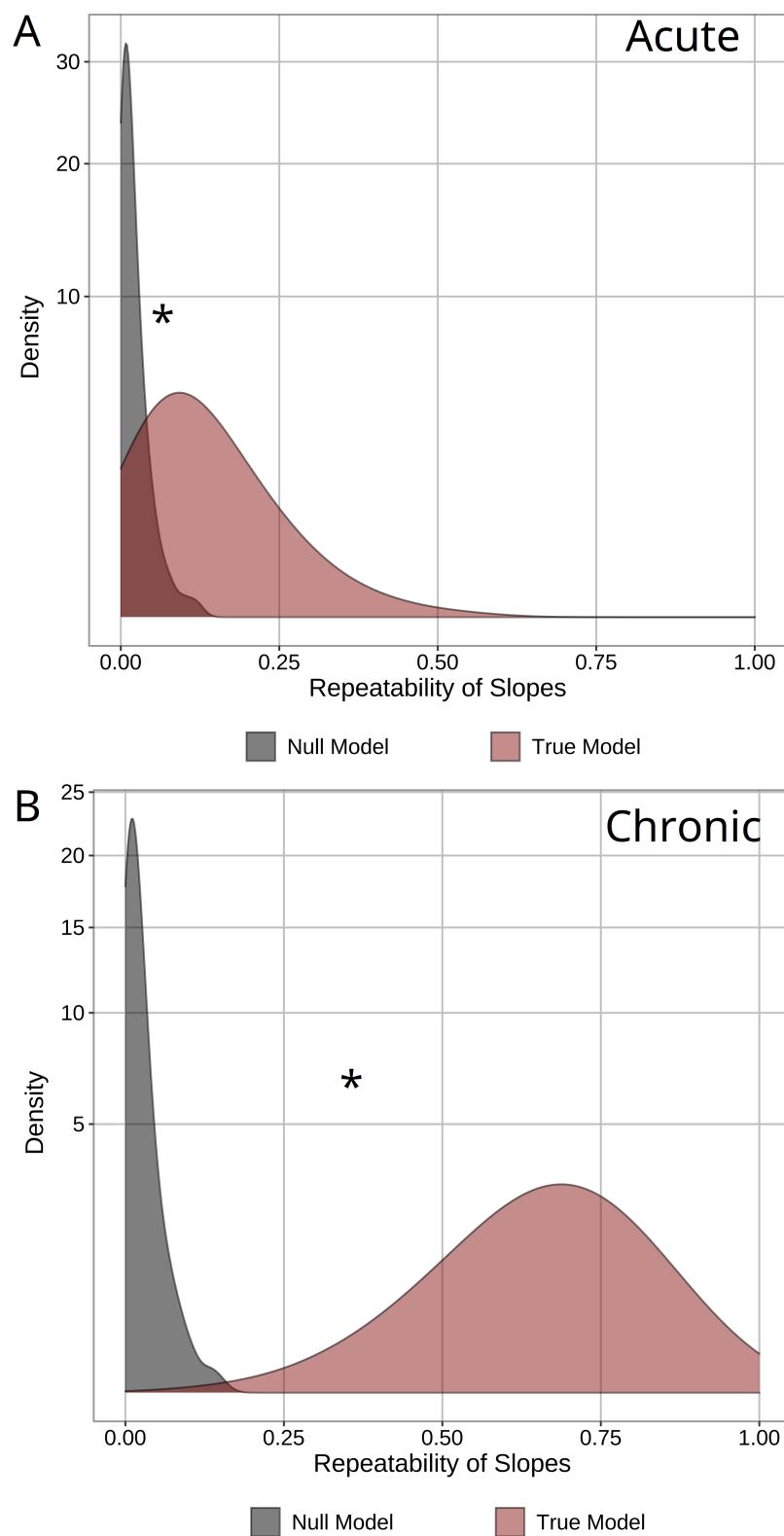


FIGURE 5 Repeatability of acute and chronic changes in dry heat transfer ( $q_{Tot}$ ) at the eye region during stress exposure in black-capped chickadees ( $n = 19$ ). Panels A and B represent distribution of repeatability values for acute and chronic responses to stress exposure, respectively. True model distributions (red) represent those drawn from models where identity of individuals was correctly identified. In contrast, null model distributions (grey) represent those drawn from models where identity of individuals was randomly scrambled. A positive difference between true and null distributions (indicated by an asterisk, "") implies that repeatability values from true models cannot be explained by biases in experimental methods (captured in null models) and are considered significant. Distributions are estimated from posteriors of Bayesian generalised additive mixed effects models (GAMM). Thermal responses to stress exposure represent those observed at the eye region of chickadees, using infra-red thermography across 60 days of observation.

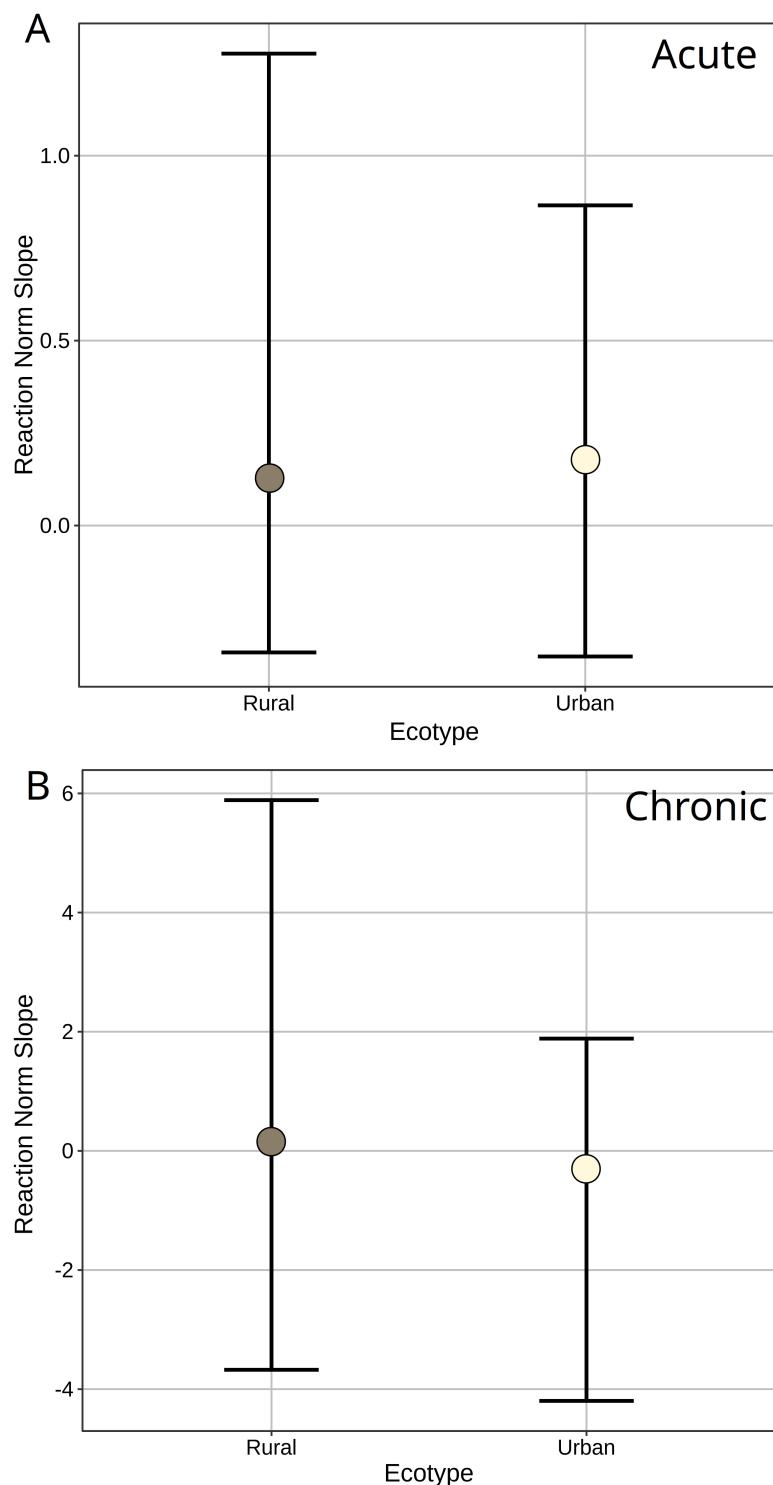


FIGURE 6 Average effect of stress exposure on dry heat transfer ( $q_{Tot}$ ; reaction norm slopes) at the eye region of black-capped chickadees ( $n = 19$ ) captured from urban and rural ecotypes ( $n = 9$  urban,  $n = 10$  rural). A | Average slopes of acute reaction norm across individuals captured at each ecotype. Reaction norm slopes represent the slopes of the linear interaction between treatment type and time post stress exposure (s) per individual. B | Average slopes of chronic reaction norms across individuals captured from each ecotype. Here, reaction norm slopes represent those of linear interactions between treatment type and ambient temperature ( $^{\circ}\text{C}$ ) per individual. Error bars represent 95% credible intervals around mean estimates. All reaction norm slopes were derived from Bayesian generalised additive mixed effects models (GAMMs).

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